**Remarks**

Applicants have rewritten claim 4 in independent form. Claim 17 has been amended to perfect the antecedent basis of step (c). The amendments are clerical. Applicants have added new claim 20. Support for the claim can be found, for example in paragraph [015]. Accordingly, no new matter has been introduced by the amendment or the new claim and their entry is respectfully requested.

Applicants now turn to the specific rejections.

The Examiner rejected claims 1, 4 – 6, and 8 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano et al. (1990) ("Ruano") in view of Furlong et al. (1993) ("Furlong") and Ross et al (1998)("Ross"). The Examiner contends that "it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the haplotype analysis methods of Ruano et al in view of Furlong et al so as to incorporated [sic] the primer extension/mass spectrometry based genotype detection methods of Ross et al." The Examiner further contended that one would have been motivated to combine Ross with Ruano and Furlong because Ross allegedly teaches that "primer extension/mass spectrometry based methods eliminate excess handling and can resolve many possible genotypes/loci using a single non-fluorescent primer."

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

Applicants respectfully submit that contrary to the Examiner's conclusion, one skilled in the art would not have combined Ruano and/or Furlong with Ross. This is because the method described by Ross is simply not applicable to haplotyping analysis and Ross uses a fundamentally different approach than Furlong and Ruano. Ross does not use a single nucleic acid dilution which is a critical starting material for the methods of the present invention. Rather, Ross uses a significant concentration of starting material, namely 25 ng of genomic DNA per 25 µl reaction (see, p. 1350, col. 2), i.e., a robust amount of the nucleic acid being amplified. As previously discussed and as well known to a skilled artisan, PCR amplification is prone to errors. These errors are particularly magnified in amplification of dilute samples as opposed to

using a robust amount of nucleic acid as a starting material. Given the sensitivity of the Ross method, such errors would be expected to be magnified. However, if one uses a detection method that is less sensitive to detection of errors, such as gel electrophoresis as taught by Ruano and Furlong, one can avoid being overwhelmed by the background noise from such PCR errors. However, using a very sensitive detection method such as mass spectrometry the artisan would expect such technique to be subject to much more background noise because of detection of the errors as well as the intended peaks. Ross did not see this because he used a robust amount of original nucleic acids which compensated the detection or errors made during PCR. Therefore, a skilled artisan would not have looked to combine Ross with the methods of Ruano and Furlong. This is because Ruano and Furlong use a dilute starting sample as opposed to a robust starting sample as in Ross. Even assuming *arguendo* that a skilled artisan would have done so, a skilled artisan would not have expected the method to provide improvement in view of the known qualities of PCR and mass spectrometry.

Moreover, to overcome the less efficient amplification of a dilute sample, Ruano used a so called booster PCR, in which the sample is amplified two times. This not only added an additional step to the presently claimed method, but it also results in introduction of even more errors into the reaction mixture. This may not cause a problem in a non-sensitive gelelectrophoresis detection, but would have been expected to result in severe background problems in mass spectrometric detection because of the well recognized sensitivity of this method. Furlong does not show a solution to this anticipated problem of introduction of PCR errors into the reaction with single molecule starting material. Furlong enhances their success by visualization of the sorted sperm to ensure that each sample has a template, and concludes that this visualization "proved important, as early experiments demonstrated that the proportion of wells amplifying was increased when sperm were sorted into the center of the well" (p. 1198, first col.). This adds an additional step to the method. In addition, because of the less sensitive detection method, Furlong is able to able to determine the molecules with varying lengths without detecting much background noise. Thus, even if a skilled artisan would have wanted to detect the products of Furlong with the method of Ross, they would expect that they had to use

the additional step of visualization, which would not have been nearly as effective a method as the presently claimed methods are.

Applicants surprisingly found that multiplex PCR of single molecule dilution can be accurately detected using mass spectrometry. Using impermissible hindsight, the Examiner reading Ross can now conclude that this was obvious because Applicants have now proved that one can use a dilute sample and mass spectrometry with high accuracy. Prior to Applicants' work and discovery, this would not have been expected by one skilled in the art. The Examiner ignores the difficulties a skilled artisan would have expected from **multiplex analysis of single molecule dilutions**. Ross does not teach or suggest that the multiplex amplification can be done, whether accurately or not, if the starting material is a single nucleic acid molecule. In contrast, Applicants specifically teach that when dealing with dilute samples efficiency of amplification of fragments is critical to allow accurate determination of genotypes and consequently haplotypes, (see, e.g. pars. [015] and [077]). Moreover, Applicants establish, for the first time, that mass spectrometry can be used to accurately detect the amplification products from a single molecule dilution. There is nothing in the combination of Ruano, Furlong and Ross that would have taught or suggested this to a skilled artisan.

Neither Ruano nor Furlong teach or even suggest using single nucleotide polymorphisms (SNPs) for haplotype analysis from single nucleic acid dilutions such as claims 4 or 6. The reasons for this is simple: such polymorphisms would not have been amenable for detection by gel electrophoresis. Given the known difficulties in PCR of single nucleic acids dilutions, a skilled artisan would not have expected analysis of SNPs using the method of Ross to work for such dilute samples. Thus, claims 4 and 6 are even more certainly not obvious. Amplification of short fragment to improve efficiency in single molecule amplification for accurate genotyping and consequent haplotyping is not disclosed in any of the cited references. Accordingly, the rejection is not applicable to claim 20.

Accordingly, the Applicants respectfully submit that the rejection of claims 1 and 4-6, 8 should be withdrawn.

Applicants respectfully submit that the rejection does not apply to new claim 20.

The Examiner rejected claims 3 and 9 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano in view of Furlong and Ross and further in view of Drysdale et al. (2000) ("Drysdale").

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, one would not have had motivation to combine Ross with Ruano and Furlong. Drysdale does not overcome this deficiency. All Drysdale describes is use of beta2-adrenegic receptor haplotypes in the prediction of response to abuterol.

Accordingly, the Applicants respectfully submit that the rejection of claims 3 and 9 should be withdrawn.

The Examiner rejected claims12 and 15-17 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano in view of Furlong and Ross, and further in view of Rein et al., (1998)("Rein").

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, one would not have had motivation to combine Ross with Ruano and Furlong. Rein does not overcome this deficiency. All Rein describes is a method for identification of 5-methylcytosine and related mutations in DNA genomes.

Accordingly, the Applicants respectfully submit that the rejection of claims 12 and 15-17 should be withdrawn.

The Examiner provisionally rejected claims 1-19 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-18 of copending Application No. 10/542,043 in view of Furlong et al (1993).

Applicants submit herewith a Terminal Disclaimer with respect to Application No. 10/542,043.

In view of the above, Applicants respectfully submit that all the claims are now in condition for allowance. Early and favorable consideration is earnestly solicited.

Should any fee deficiencies be associated with this submission, the Commissioner is authorized to debit such deficiencies to the Nixon Peabody LLP Deposit Account No. 50-0850. Any overpayments should be credited to said Deposit Account.

Date: April 7, 2008

Respectfully submitted,

/Leena H. Karttunen/

Customer No.: 50607

Ronald I. Eisenstein (Reg. No. 30,628)
Leena H. Karttunen (Reg. No. 60,335)
Nixon Peabody LLP
(617) 345-6054 / 1367